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Bacterial Aerosolization from an Ultrasonic Cleaner

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An ultrasonic device used for cleaning surgical instruments was found to produce a significant microbial aerosol. No correlation was found between the concentration of aerosol generated and the bacterial contamination in the cleaning solution. Table top contamination around the cleaner was due essentially to splash and dripping, and not from the settling of the aerosol. Recommendations are made for cleaning and disinfection protocols.

Patients in health-care facilities share a common "sea of air." The classic airborne respiratory infections, smallpox and tuberculosis, suggest that this air environment can play a role in disease transmission. It is important to note that although the contact route is preeminently more significant, air is probably the critical environmental route.

This investigation was initiated to determine if an ultrasonic cleaner used to clean obstetrical instruments in North Carolina Memorial Hospital, Chapel Hill, N.C., produced a microbiological aerosol. The cleaning solution from this device had been cultured and found to contain a total colony count exceeding 100,000/ml and a heavy growth of *Bacillus* and *Pseudomonas* sp. Wet sites in hospitals (mops, faucets, sinks, drains, water baths) are potential reservoirs for bacteria, and in particular, *Pseudomonas*, an opportunistic pathogen capable of survival in moist environments extremely low in organic matter (9).

The most important physical action associated with ultrasonic waves propagated in a liquid medium is cavitation (3). Cavitation can be simply defined as the rapid formation and collapse of "cavities" or bubbles in the irradiated liquid. Fundamentally, the microscopic gas bubble acts as a discontinuity in the liquid structure. These microscopic, suspended gas bubbles are subject to easy rupture since the forces holding the liquid together at those points cannot resist strong tensile stresses. (7). Once a cavitation bubble forms, the void almost instantaneously implodes, due primarily to hydrostatic pressure, atmospheric pressure, and the release of surface tension. The hydraulic shock accompanying the collapse of these bubbles at the air-liquid interface creates a measurable aerosol.

The very high pressures generated by cavitation can break chemical bonds, promote oxidation, reduction, degradation and synthesis of inorganic and organic substances, and cause polymerization and intramolecular regrouping (3).

As early as 1929, Harvey and Loomis reported that the luminous bacteria *Bacillus fisheri* could be disrupted and killed in an aqueous suspension when irradiated with ultrasonic waves of frequencies around 400,000 Hz (5). Other investigations demonstrate that different bacteria exhibit different sensitivity patterns to ultrasound (2, 8). Hesselberg found that if the concentration of bacterial cells in an irradiated liquid medium was high enough ($>10^6$), cavitation was inhibited and destruction of the cells was reduced (6). The energy level and exposure time are obviously critical in the disruption of cells.

Although the mechanical forces of cavitation are the primary lethal mechanism whereby rupture and/or fragmentation of microbial cells occurs, ultrasound can also cause nonlethal and observable biochemical and functional changes, even in those organisms considered resistant to ultrasound. Changes that have been observed include increased sensitivity to disinfectants after exposure, increased ability to adsorb bacteriophage, destructive changes in and loss of capsule, and changes in the progeny of exposed bacteria (3).

Advances in the early 1950's in electrical engineering led to the introduction of the first "sonic-energy" cleaning system at the 1956 American Hospital Show in Chicago. Since then, ultrasonic cleaning devices have been used successfully in hospitals nationwide.

A basic hospital ultrasonic cleaning device consists of two fundamental components, a generator and a transducer. A 60-Hz current feeds into the generator, where it is transformed

into electrical energy of 20,000 Hz. The transducer converts the electrical energy into mechanical energy, the form of which is ultrasonic waves. The ultrasonic waves then propagate through the water bath containing the instruments. The negative pressure associated with the hydraulic shock loosens and/or removes soils attached to the surface of objects placed in the cleaning solution.

MATERIALS AND METHODS

The ultrasonic cleaning device investigated in this study is used for cleaning postoperative obstetrical instruments prior to sterilization in the Obstetrics-Gynecology Unit of North Carolina Memorial Hospital. The cleaner was manufactured by the A. S. Aloe Co. (Aloe-Narda Sonic-Cleaner, St. Louis, Mo.). The overall dimensions of the tank were 36 by 25 by 33 cm, and the water bath contained 10.2 liters of cleaning solution when in operation. The cleaner operates at a frequency of 40,000 Hz, a power of 200 W, and a plate current of 44 volts. The detergent used, Robark Surgisolvent, is a product of Roane-Barker, Inc., Raleigh, N.C.

Air sampling. Airborne microbial-bearing particles generated and dispersed from the liquid surface of the ultrasonic cleaner were collected on Trypticase soy agar (Baltimore Biological Laboratories, Baltimore, Md.) in a model FD-100 air slit sampler (Reyniers and Son, Chicago, Ill.) (10). The width of the slit was set at 0.152 mm and adjusted to 1.5 mm above the agar surface. A flow meter was used to maintain a rate of 1 ft^3 (about .028 m^3) per min. This instrument has a collection efficiency of 85 to 95% of the total aerosol present (M. D. Decker, Bacterial air samplers, U. S. Army Chemical Corps, Frederick, Md., 1958, p. 7).

The air above the water surface of the cleaner was sampled with and without a plexiglass tank cover. Samples collected without the cover represented microbial particles generated by the cleaner as well as those contributed by the room air. With cover in place, all the particles collected were assumed to be those generated by the cleaner. To avoid creating a vacuum great enough to burst detergent bubbles, the cover was not designed to fit tightly (Fig. 1).

Ten samples, each representing 60 cubic feet of air (1 cubic ft per min for 60 min), were collected with the cover in place and 10 samples were collected with the cover removed. The first 15 min of each sampling period (60 min) established background counts with the ultrasonic cleaner off. At the end of 15 min either contaminated or sterile instruments were placed in the cleaner. After a 5-min soak period, the cleaner was turned on and operated for 25 min. When a total sampling time of 45 min had elapsed, the cleaner was turned off and air sampling was continued for the final 15 min. Culture dishes containing the sample were incubated for 24 to 30 h at 35 C, colonies were counted, and the number of colony-forming units per ft^3 of air was recorded.

Surface sampling. Disposable plastic contact plates containing Trypticase soy agar with lecithin and polysorbate 80 were used to sample nine sites on the counter top around the ultrasonic cleaner before and after each air sampling run. Contact plates were incubated overnight at 35 C, counted, and recorded as the number of colony-forming units per 4 $inch^2$ of surface area.

Microbial contamination of cleaning solution. A 10-ml sample of cleaning solution was collected with a

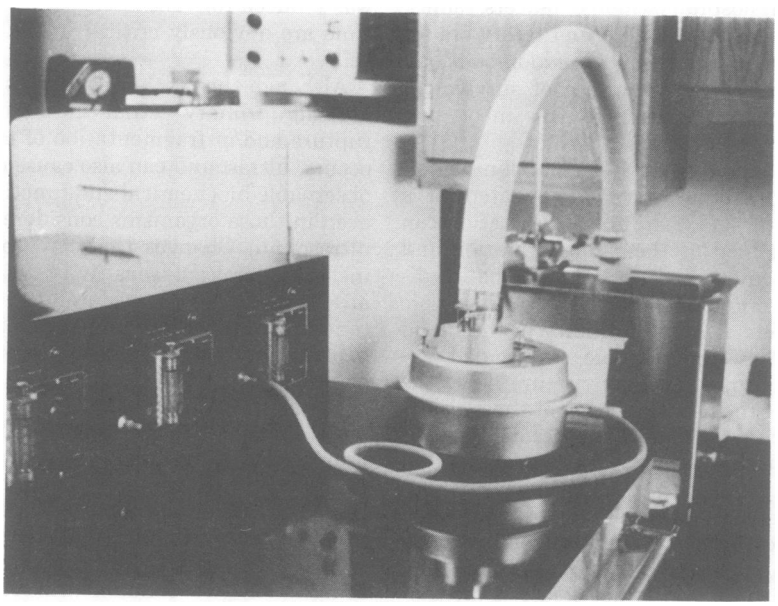

FIG. 1. *Ultrasonic cleaner and air sampler.*

sterile pipet before and after each air sampling run. Each sample was placed in 90 ml of sterile phosphate buffer containing 0.5% polysorbate 80 (Tween 80) and was thoroughly mixed. Appropriate dilutions were made in the same buffer and pour plates were prepared by using standard methods plate count agar. Plates were incubated at 35 C for 24 h, and the results were recorded as the total colony count per ml of cleaning solution. Each dilution was also plated in Pseudosel Agar (BBL) and examined after incubation under ultraviolet light for fluorescence to determine the presence or absence of *Pseudomonas aeruginosa*.

RESULTS

Tables 1 and 2 show the concentration of bacterial aerosols produced by the ultrasonic cleaner with and without the cover. Contamination of the cleaning solution before and after each sample run is also shown in the tables. With the cover in place on the cleaning tank, the number of colony-forming units in the air increased as much as 255 times over the initial background count per cubic foot of air and with the cover removed, counts increased as much as 28 times over the initial background. A Student's *t*-test (degrees of freedom = 9) was performed on the difference of the log of the means between background and in-operation counts, with and without the cover. The increase in mean air counts above background was highly significant ($P = 0.006$ with cover; $P = 0.0006$ without cover).

Table 3 indicates bacterial contamination of the counter top around the ultrasonic cleaner. The counts at any one sampling site varied from 1 to more than 300 per 4 inch^2 of surface. Surface contamination was not significantly affected by the aerosol produced.

There was no apparent correlation between the aerosol concentration and the number of bacteria in the cleaning solution. Correlation

TABLE 1. *Water and air contamination of a sonic cleaner without the cover*

Sample	Mean air contamination (CFU/ft^3)[a]				Cleaning solution contamination (counts/ml)	
	Background (0 to 15 min)	Instruments (16 to 20 min)	Aerosol (21 to 45 min)	Background (46 to 60 min)	Before cleaning	After cleaning
1	1.67	1.00	14.80	0.20	150	500
2	1.93	0.20	4.64	0.73	130	200
3	1.07	1.20	5.48	1.33	>400,000	>400,000
4	0.33	0.40	5.84	0.33	>100,000	>100,000
5	2.27	2.40	1.52	1.33	150	500
6	0.80	1.40	1.40	0.60	280	310
7	0.60	0.80	20.68	1.07	20	30
8	0.27	0.20	5.64	1.00	125	100
9	1.53	1.00	9.88	1.20	70	100
10	0.33	0.00	1.08	0.27	900	1,500

[a] Each mean is the average of counts per minute during the particular time period. CFU, Colony-forming unit.

TABLE 2. *Water and air contamination of a sonic cleaner with the cover*

Sample	Mean air contamination (CFU/ft^3)[a]				Cleaning solution contamination (counts/ml)	
	Background (0 to 15 min)	Instruments (16 to 20 min)	Aerosol (21 to 45 min)	Background (46 to 60 min)	Before cleaning	After cleaning
1	1.13	1.00	215.04	1.47	30	80
2	1.67	2.20	1.48	1.07	2,000	3,000
3	1.07	0.80	0.96	0.27	20	200
4	2.07	3.00	4.64	1.00	300	300
5	1.80	0.60	2.16	0.47	100	200
6	0.60	3.20	300.00	0.53	<10	100
7	0.80	1.00	255.32	0.33	1,500	2,000
8	0.87	1.60	2.44	0.60	60	130
9	1.87	1.00	7.64	0.60	60	300
10	0.80	0.80	4.52	0.27	<10	<10

[a] See footnote to Table 1.

coefficients were computed for the bacterial concentration in the air and cleaning solution. With the cover in place over the tank, the coefficient was −0.090 before the cleaning cycle and −0.057 after the cleaning cycle. With the cover removed, the coefficients were −0.107 and −0.108.

Approximately 5% of the colonies on each air sample plate were picked and streaked on a Pseudosel Agar plate and stabbed into a Pseudosel slant. After incubation at 35 C, plates and slants were examined for pyocyanin production and fluorescence under ultraviolet light. This procedure clearly demonstrated that *P. aeruginosa* was aerosolized from the cleaning bath. No attempt was made to identify other species in the bacterial aerosol.

TABLE 3. *Contamination of counter top*

Sample	Mean no. of colonies per 4 inch²[a]	
	Before operation of cleaner	After operation of cleaner
1	3.0	6.2
2	6.6	15.6
3	12.1	18.7
4	41.6	18.6
5	16.1	13.1
6	57.6	25.0
7	16.1	15.1
8	17.2	13.0
9	7.7	10.2
10	17.1	15.1

[a] Each mean is the average of nine samples.

DISCUSSION

A significant microbial aerosol was produced by this ultrasonic cleaner (Fig. 2). Anyone using these devices should recognize that they can be a source of airborne microorganisms. However, the potential hazard of this transmission route for nosocomial diseases has not been adequately assessed.

The microbial contamination of the counter top around the ultrasonic cleaner was not significantly affected by the aerosol generated. If the aerosol particles were very small, these bacteria would become part of the room air flora. The primary source of contamination of counter tops was apparently spillage and dripping from the instrument basket during introduction and removal of instruments from the cleaning solution.

The lack of correlation between air and water contamination is probably attributable to many unquantified variables. These factors include: (i) Instrument load: the number and arrangement of the instruments could cause varying degrees of deflection of the ultrasound and thereby influence cavitation effects. Instruments not completely submerged in the cleaning solution could disrupt the capillary wave atomization mechanism at the air-liquid interface and cause variations in the rate, size, and amount of aerosol generated. (ii) Microbial flora of the cleaning solution: the size and other morphological characteristics of individual species could affect the generation of an ultrasonic aerosol. (iii) Detergent used in cleaning solu-

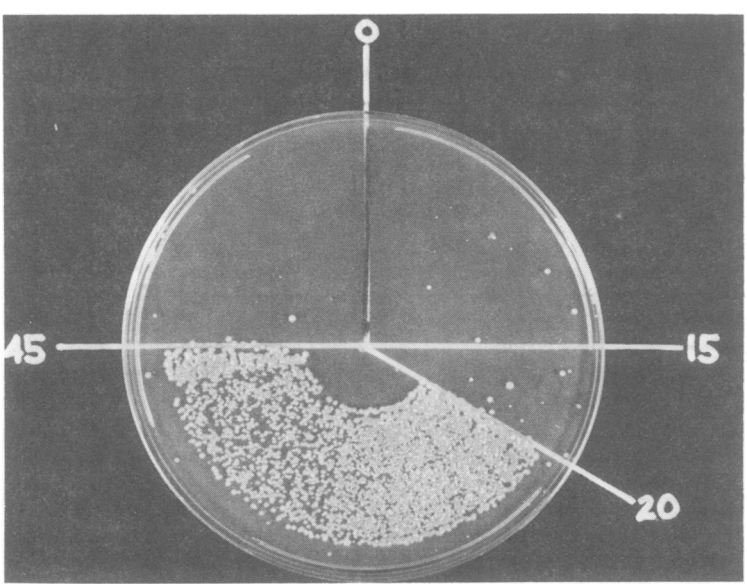

FIG. 2. *Air sample plate. Time intervals are shown; 20 to 45 min represents the ultrasonic cleaning cycle.*

tion: the amount and type of detergent used could affect atomization. The emulsifying nature of detergents and their ability to lower the surface tension of water could alter intermolecular bond strength and influence the formation of cavitation bubbles. (iv) Temperature and dissolved gasses: these must also be considered. Investigation of these factors is continuing.

Ultrasonic cleaners can be used safely and airborne contamination from this source can be minimized by daily cleaning of the solution tank, adding a germicide to the cleaning solution, and placing a cover over the tank while cleaning is in progress (1).

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